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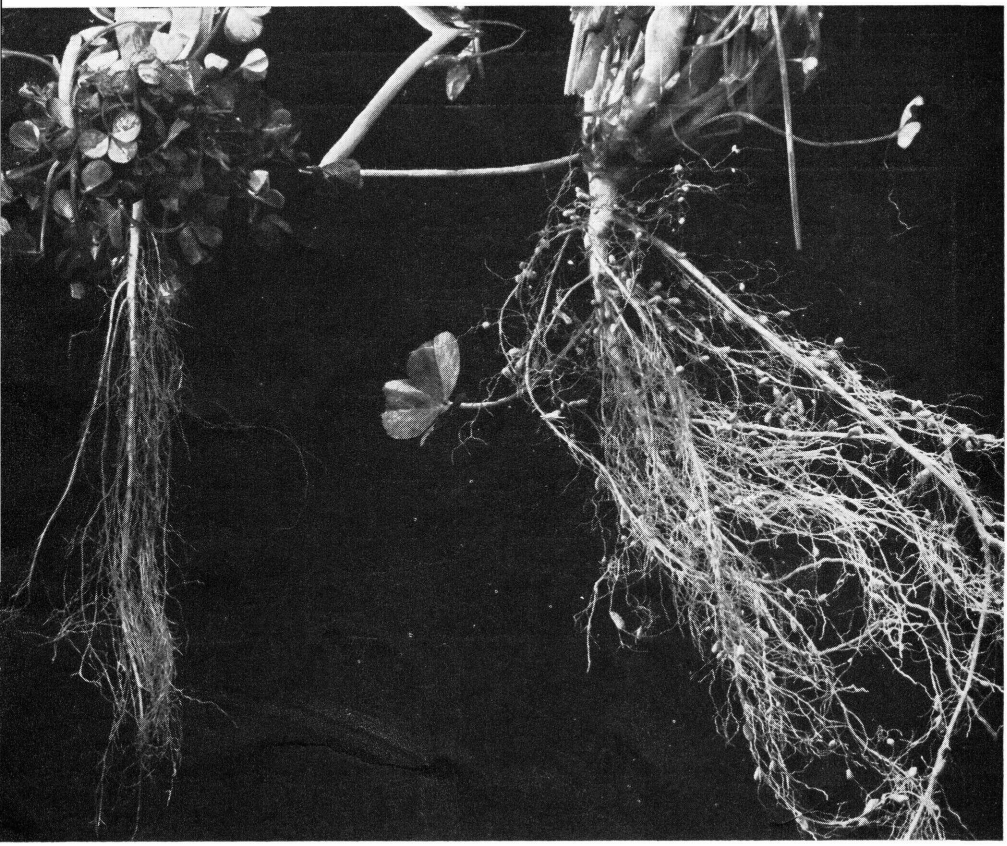
LEGUME INOCULATION



• WHAT IT IS
• WHAT IT DOES

Farmers' Bulletin No. 2003

UNITED STATES DEPARTMENT OF AGRICULTURE

CONTENTS

For Successful Legume Inoculation—

Use the right inoculant for the legume.

Keep commercial culture in cool, dark place until used.

Follow directions and mix culture well with seed.

Plant seed within 48 hours after inoculated, or reinoculate.

Inoculate in all cases of doubt and always on new land.

Prepare a good, well-fertilized, moist seedbed; after planting small seeds, cultipack the soil.

BN-6986-X

Illustration on cover shows specimens of crimson clover roots. Properly inoculated seed produced vigorous growth and extensive nodulation. The specimen on left was grown from uninoculated seed.

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LEGUME INOCULATION: WHAT IT IS - WHAT IT DOES

By Lewis W. Erdman, principal bacteriologist, Soil and Water Conservation Research Division, Agricultural Research Service

Nitrogen is indispensable to life because it is the key ingredient in protein.

The air we breathe is primarily a mixture of nitrogen and oxygen gases. About 80 percent by volume is pure nitrogen in a free, or uncombined, state. Every acre of land surface has about 35,000 tons of this free nitrogen above it.

Free nitrogen, as such, is useless to plant or animal life. To become useful, it must enter into combination with other elements. But it does not combine with other elements merely by coming in contact with them. It is forced into combination by powerful influences such as lightning and reactions brought about by tremendous heat.

NITROGEN FIXATION

Farmers can obtain atmospheric nitrogen for their crops by growing inoculated legumes. The inoculating process consists of mixing legume seeds with the correct strain of bacteria culture before the seeds are planted.

Soon after the legumes begin to grow, the legume bacteria invade the root hairs. They multiply in large numbers. The legume forms growths called nodules (fig. 1). The bacteria live in these nodules and do their beneficial work.

A definite partnership is established. The legume plant furnishes the necessary sugar or energy. The bacteria use this energy to change the free nitrogen of the atmosphere into a form that the plant can assimilate and use to build protein. The nitrogen is said to be *fixed.*

This beneficial association was discovered in 1886. In 1901 the United States Department of Agriculture began its investigations on methods of promoting it. Since then the Department has given farmers accurate information on how inoculation increases legume yields. It has pointed out to scientific workers the precautions they must take to prepare satisfactory inoculant cultures.

The quantity of nitrogen taken from the air and fixed by the legume bacteria for different legumes is

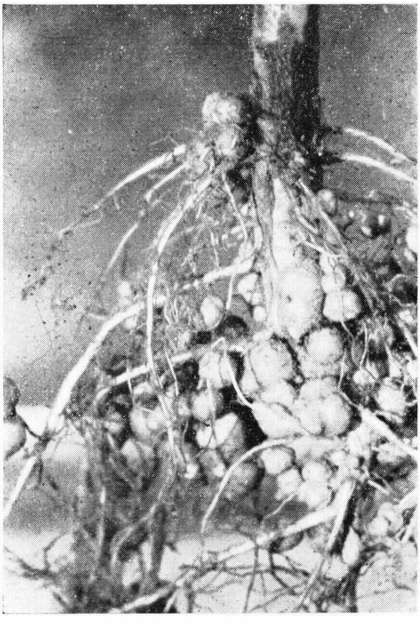

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Figure 1.—Highly effective nodulation on soybean roots. Clusters around the taproot were produced by inoculant added to seed.

difficult to calculate. It varies with (1) the kind of legume, (2) the effectiveness of the legume bacteria, (3) the soil conditions, and (4) the presence of necessary plant-food elements exclusive of nitrogen. In high-fertility soils, well supplied with available nitrate nitrogen, little or no fixation may occur, as the legume plants seem to use this available nitrogen rather than encourage the bacteria to fix more. Most noticeable results from legume inoculation are obtained on soils of average fertility or on depleted soils.

Those factors, exclusive of nitrogen, that make for the optimum growth of legumes play an important part in increasing the quantity of nitrogen that is fixed by legumes. Usually a combination of growth factors reacting favorably governs how much nitrogen is fixed. Although it is not possible to determine exactly how much nitrogen is fixed by the bacteria in the nodules on legume plant roots, different investigators have reported their findings on the most important legumes:

Legume:	*Average amount of nitrogen fixed per acre (pounds)*
Alfalfa	194
Ladino clover	179
Lupines	151
Sweetclover	119
Alsike clover	119
Red clover	114
Kudzu	107
Legumes in pastures	106
White clover	103
Lentils	103
Sourclover	98
Crimson clover	94
Cowpeas	90
Lespedezas (annual)	85
Fenugreek	82
Vetch	80
Bur-clover	78
Peas	72
Velvetbeans	67
Garbanzo	66
Soybeans	58
Winter peas	50
Peanuts	42
Beans	40

The cost of the 1-bushel-size inoculant unit for alfalfa and clovers is about 50 cents. This size inoculates 3 to 5 acres, depending on the rate of seeding. It is therefore possible for a legume grower to get 970 pounds of nitrogen for only 50 cents.

THE LEGUMES

Legumes are used for hay, silage, seed, winter cover crops, and pasture.

Of the more than 10,000 known species of legumes, only about 200 are cultivated by man. In the United States only about 50 are grown commercially. These species further divide into varieties. For instance, more than 100 named varieties of soybeans are being grown in this country.

The legumes are rich in high-quality protein. They are well supplied with phosphorus and calcium. They are a good source of vitamins, especially vitamins A and D. These qualities make legumes one of man's best foods. They are almost indispensable for efficient, economical livestock feeding.

The protein in legumes is directly related to high nitrogen content. In this respect they differ markedly from grasses and other nonlegumes.

For example, the average protein content of 1 ton of each of 8 legume hays was compared with the protein in 8 grasses. The legumes averaged 304 pounds of protein per ton and the grasses 156 pounds.

PURPOSE OF INOCULATION

The fundamental purpose of legume inoculation is to add a fresh culture of effective strains of legume bacteria to the soil or to the seed—preferably to the seed. When the young plant begins to grow, the bacteria are right there to enter the root hairs and to begin their beneficial work. Figure 2 illustrates the effectiveness of proper inoculation.

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Figure 2.—Soybean plant on left was grown from uninoculated seed. Other plants were grown from seed properly inoculated with effective soybean bacteria.

Effective inoculation of legumes has been a major factor in improving their yield and quality. Legume inoculation makes available greater quantities of high-protein feeds so necessary in livestock production. Legumes add nitrogen to the soil. Their growth contributes to the maintenance of good-quality organic matter. Organic matter improves the physical property of the soil, increases its moisture-holding capacity, and helps it hold plant nutrients. The combination of organic matter and necessary fertilizers provides a readily available supply of plant nutrients for crop production.

Data show that the more effective strains of legume bacteria can increase the yield or protein content of legumes as much as 20 percent on the average over the natural legume bacteria in the soil.

Too often it is taken for granted that inoculation is not necessary because a legume has been grown in the same soil. If the proper bacteria are not present the young legume plants look spindling and sick (fig. 3). Such cases present a real problem because it is more difficult to get growing plants inoculated than it is to inoculate the seeds before planting.

NEED FOR INOCULATION

Not all agricultural soils contain the bacteria necessary to promote successful growth in legumes. Furthermore, many of the legume bacteria naturally present in cultivated soils are not high nitrogen-fixing strains (figs. 4 and 5). For example, 100 soybean fields in Wisconsin were examined and the soybean bacteria isolated. When these strains were tested, 25 percent proved to be effective, 50 percent average, and the rest poor or ineffective.

In Kansas, 217 strains of alfalfa and sweetclover bacteria were isolated from growing plants within a restricted area. When these strains were tested, 27 percent fixed a high

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Figure 3.—Left, effective yellow lupine inoculation; right, uninoculated.

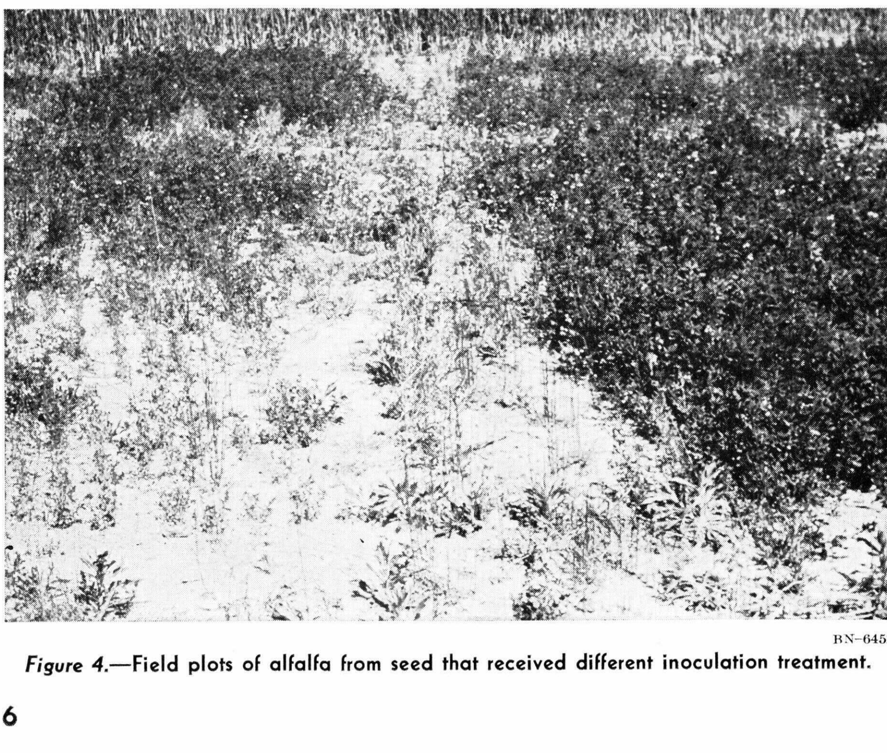

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Figure 4.—Field plots of alfalfa from seed that received different inoculation treatment.

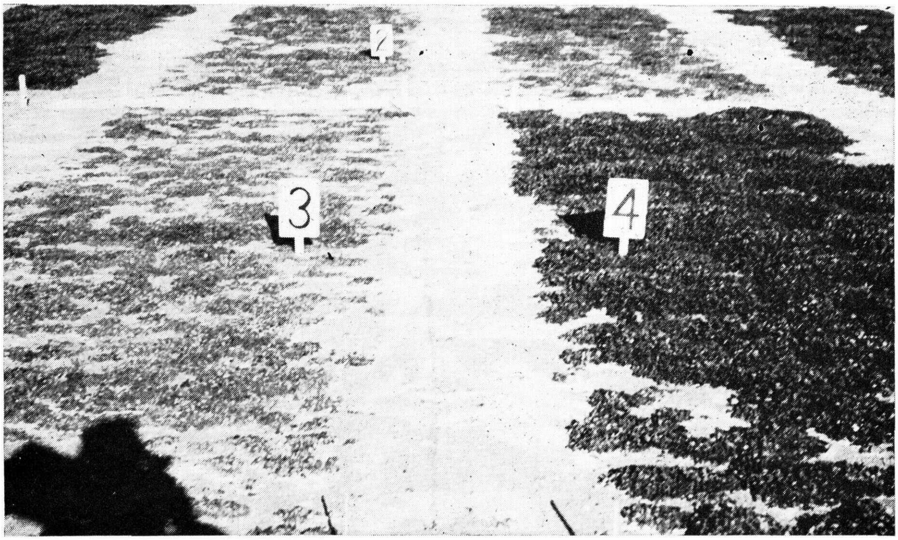

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***Figure 5.*—Field plots of crimson clover from seed that received different inoculation treatment.**

amount of nitrogen, 21 percent fixed a small amount of nitrogen, and 52 percent fixed in-between amounts of nitrogen.

A survey for different States indicates that more than 75,000,000 acres are planted each year with some legume. There is no way of telling exactly what percentage of these legume seeds are inoculated. If an estimate is based on the total number of cultures sold, probably less than 20 percent, or about 15,000,000 acres, are being inoculated.

On the remaining 60,000,000 acres, thousands of acres contain strains of legume bacteria capable of thoroughly inoculating the legumes planted in these soils. Farmers who have been growing legumes in such soils for long periods generally know when it is not necessary to inoculate legume seeds. On the other hand, many farmers are not sure whether it pays to inoculate. Certainly there is every reason to believe that in many sections of the country many acres of land need effective strains of legume bacteria to bring about the maximum benefits from legume crops. The facts offer a challenge to legume growers. The gains possible from the more productive strains of legume bacteria should persuade nearly every farmer to make a practical test in his own field.

LEGUME BACTERIA

Legume bacteria are single-celled micro-organisms that vary in size and shape with age and with the composition of the medium in which they grow. Under the ordinary microscope with a magnification of 1,000 diameters they may be either the usual rod forms, 0.5 to 0.9 micron wide and 1.2 to 3 microns long (a micron is 1/25,000 inch), or the irregular, club-shaped forms shown in figure 6.

When legume bacteria are young, they are extremely active; they have been observed to have either one polar flagellum (propeller organ) or a number of flagella surrounding the cell.

How Bacteria Work

Just how legume bacteria work is still unknown. Apparently, however, they work with remarkable ease. Experiments have given evidence of nitrogen fixation after 2 or

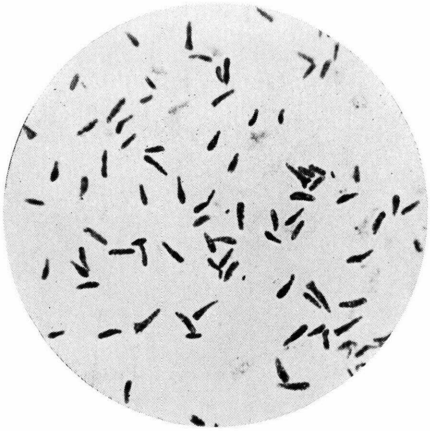

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Figure 6.—**Legume bacteria seen through a powerful microscope.**

3 weeks. A deeper, darker green in inoculated legumes is one sure sign of nitrogen fixation by the bacteria.

Nodules and Nitrogen

Inoculated legumes growing in normal soils display definite characteristic types of nodule formation. Some of these types are shown in figure 7.

The clustering of nodules around the taproot at the point where the inoculated seed is planted generally indicates that the nodules were formed by the bacteria added in the inoculant. If the inside of the nodule is red, this indicates high nitrogen-fixing activity. Nodules scattered over the side roots are usually formed by the legume bacteria naturally present in the soil.

As the legumes mature, the nitrogen compounds formed in the nodules furnish nitrogen for the building of proteins in the leaves, stems, and seed. In the early stages the nodules may contain 5 to 8 percent nitrogen, but at seed maturity they are no richer in nitrogen than the rest of the root. The nodules disintegrate rapidly at the time of seed formation.

CULTURES

Cultures represent large numbers of bacteria that are encouraged to grow and reproduce in specially prepared mediums. Their preparation and use are discussed in the following sections.

Cultures of legume bacteria are alive. Treat them carefully. They tolerate low temperatures much better than high. Exposing them to heat that is unbearable by man, or even uncomfortable, may impair or destroy their effectiveness. Store them in a cool place until used.

Although legume bacteria tolerate sunlight to some degree, avoid unnecessary exposures either of the unopened containers or of seed that has been treated. Bacteria that dry on seed soon die. If inoculated seed must be kept as long as 48 hours, reinoculate. For this reason the purchase of preinoculated seed is not advisable.

Seed treated with legume bacteria should not come in direct contact with caustic lime or mixed fertilizers. Inoculated seed may be drilled down the same spout with superphosphate or basic slag without injury to the bacteria. If the concentration of fertilizer does not injure seed germination it will not ordinarily harm legume bacteria.

Most seed disinfectants are toxic to legume bacteria. Consequently, legume seed that has been treated with disinfectant compounds should not be inoculated in the usual manner. In large scale operations, the inoculum is mixed with wheat middlings, sawdust, or other inert material and drilled before planting. This practice, called "preplanting" of the bacteria, is successful in large pea-growing areas where it becomes necessary to treat the seed.

Bacteria in Soil

If the chemical reaction of the soil is suitable, if sufficient moisture and plant food are available, and if temperatures are not too high, the

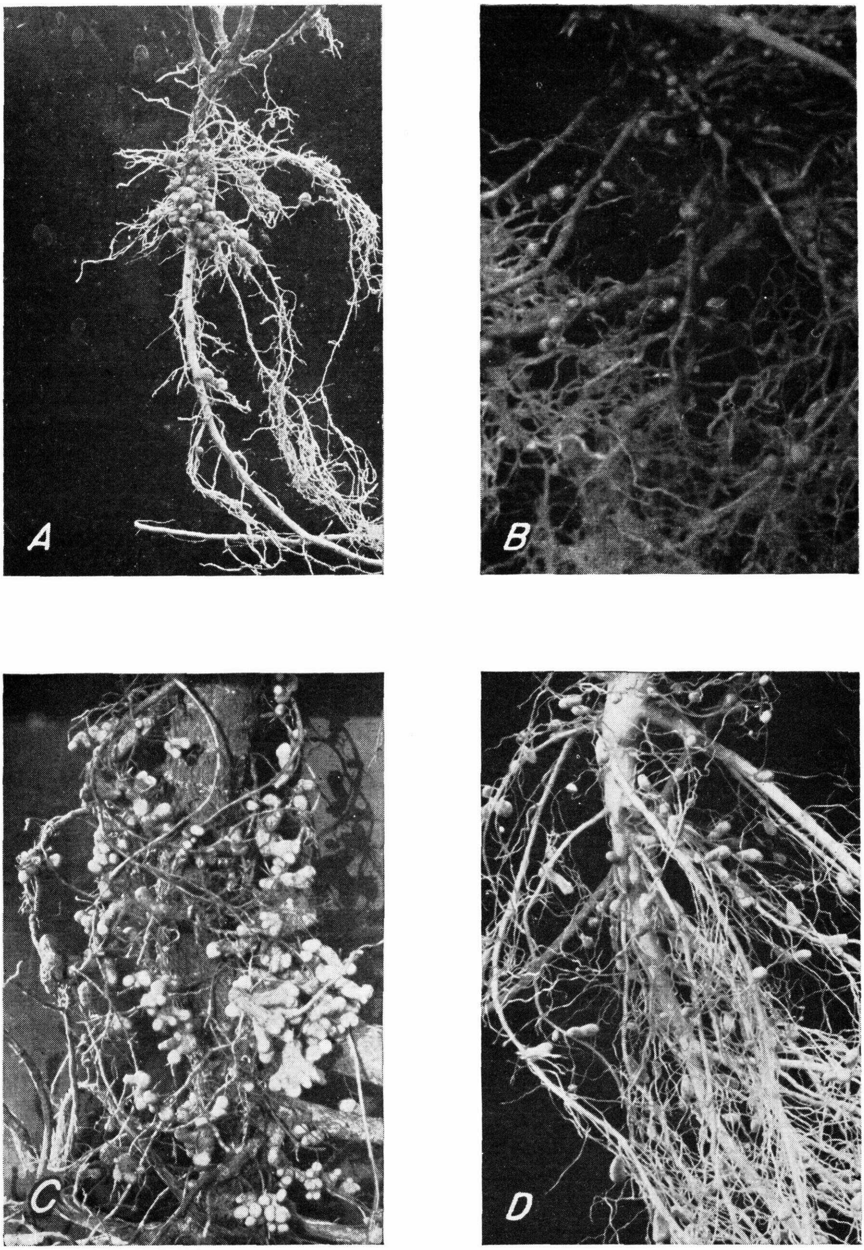

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Figure 7.—Types of nodulation produced by effective strains of bacteria on different legumes: *A*, Lespedeza; *B*, trefoil; *C*, black locust; and *D*, crimson clover.

bacteria should function normally. Acid soils sometimes affect bacteria. Bacteria of alfalfa, sweetclover, and red clover are among those that are not very acid-tolerant. Soybean, velvetbean, cowpea, vetch, lespedeza, and lupine bacteria belong to the acid-tolerant types.

In the natural competition between inhabitants of the soil, the legume bacteria have the advantage because they are protected from time to time in nodules. Without this association, they would have adverse soil conditions with which to cope.

Conditions essential to the satisfactory growth of legumes must be fulfilled before maximum results with inoculation can be expected. The principal requirements are the proper preparation of the soil; adequate moisture; the presence of an available supply of lime, phosphorous, and potash; and the use of healthy adapted viable seed.

Preparation

To prepare effective cultures for legumes, one must have specialized training and experience. Adequate laboratory facilities, equipment for controlled production, and greenhouse space or other suitable means for testing plants are prerequisites to satisfactory production.

Routine work in such a laboratory calls for periodic tests and transfers of all strains of legume bacteria used in the production of commercial inoculants. These tests are made for purity and for effectiveness on the growing plants. New strains are isolated each year, and this is an important feature of the work. These must be purified and tested also. A selection is made of the most effective strains, and a given number are used for the production of the different culture groups. For example, 5 or 6 strains may be used for a culture to inoculate the alfalfa group whereas 10 to 12 strains may be used in the production of a soybean inoculant.

The bacteria are grown either in liquids or on the surface of agar. Heavy suspensions of bacteria containing an excess supply of food are used for mixing with the carrier, which may be a finely ground peat, or mixtures of peat and charcoal, peat and sand, or other materials.

The three types of carriers generally used are: (1) moist humus or finely ground peat, (2) agar, and (3) liquid. The bulk of commercial inoculants are prepared in moist humus. Most seed suppliers handle one or more brands of commercial legume inoculants and thus make them readily available to the consumer trade. *Because of the perishable nature of these living bacteria, dealers are cautioned not to store the cultures in places that are either too warm or too dry.*

Use

Be sure that the culture is prepared for the specific seed you wish to plant, and use it before the expiration date.

In using agar cultures, add a small quantity of clean cool water to the bottle, shake it vigorously to get the bacteria in suspension, add more water, and then pour the bacterial suspension on the legume seeds and mix them until all are moistened.

In using peat or humus-type inoculants, either (1) moisten the seeds with water and empty the contents of the container on them, mixing until all are coated with the black substance; or (2) add a specified quantity of water to the inoculant to form a thin paste and pour it on the seeds.

Specific directions for using commercial cultures are found on the label of each container.

A thorough mixing is essential. Do not use too much water. Avoid soaking the seeds.

Water causes the inoculants to stick to seeds better than when inoculants are used without water.

Inoculant retention on dry soybeans averages about 8 percent; on wet soybeans, 83 percent; on dry clover, 31 percent; and on wet clover, 86 percent. If a given quantity of a soybean inoculant is satisfactory when applied without water the same quantity would be equally satisfactory for up to five times as much soybean seed if applied with water. For red clover, inoculant requirements would be cut approximately in half by using wet inoculation.

Plant the seeds as soon as possible after they are inoculated. Ideal conditions prevail shortly before a gentle rain. When inoculated small legume seeds remain on or near the surface of the soil, exposed to hot, drying winds for several weeks, supplemental inoculation is advisable. This may be done by mixing a legume inoculant with cottonseed meal, wheat middlings, or even sand and broadcasting the mixture over the soil immediately before or after a rain.

When young legume plants show lack of proper inoculation it may be desirable to reseed the area with inoculated seeds. The bacteria added on the seed may eventually gain access to the root hairs of the growing plants and produce successful inoculation.

Planting in Dry Soil

Planting inoculated seeds in dry soil is usually not recommended. However, if an adhesive, like syrup, is used to mix the inoculant with the seed, the life of the bacteria can sometimes be maintained for 2 to 3 weeks. When a 10-percent syrup solution was used on alfalfa seed planted in dry soil in September in North Carolina, 1,915 pounds of dry hay were obtained from the experimental plot. When water alone was used under the same conditions, only 1,040 pounds of hay were harvested. If rain does not come before the 2-to-3-week period has elapsed, it is always advisable to reinoculate the soil.

Group Designations

Farmers have been accustomed to ordering legume cultures according to group designations, such as the alfalfa group, the clover group, and the pea and vetch group. Seven of these groups are now recognized. They are given below, with a list of the most important legumes in each.

ALFALFA GROUP

Common name	Scientific name
Alfalfa	*Medicago sativa*
Buttonclover	*M. orbicularis*
California bur-clover	*M. denticulata*
Spotted bur-clover	*M. arabica*
Black medic	*M. lupulina*
Snail bur-clover	*M. scutellata*
Tubercle bur-clover	*M. tuberculata*
Little bur-clover	*M. minima*
Tifton bur-clover	*M. rigidula*
Yellow alfalfa	*M. falcata*
White sweetclover	*Melilotus alba*
Hubam sweetclover	*M. alba annua*
Yellow sweetclover	*M. officinalis*
Bitterclover (sourclover).	*M. indica*
Fenugreek	*Trigonella foenumgraceum*

CLOVER GROUP

Alsike clover	*Trifolium hybridum*
Crimson clover	*T. incarnatum*
Hop clover	*T. agrarium*
Small hop clover	*T. dubium*
Large hop clover	*T. procumbens*
Rabbitfoot clover	*T. arvense*
Red clover	*T. pratense*
White clover	*T. repens*
Ladino clover	*T. repens* (*giganteum*)
Sub clover	*T. subterraneum*
Strawberry clover	*T. fragiferum*
Berseem clover	*T. alexandrinum*
Cluster clover	*T. glomeratum*
Zigzag clover	*T. medium*
Ball clover	*T. nigrescens*
Persian clover	*T. resupinatum*
Carolina clover	*T. carolinianum*
Rose clover	*T. hirtum*
Buffalo clover	*T. reflexum*
Hungarian clover	*T. pannonicum*
Seaside clover	*T. wormskjoldii*
Lappa clover	*T. lappaceum*
Bigflower clover	*T. michelianum*
Puff clover	*T. fucatum*

PEA AND VETCH GROUP

Field pea	*Pisum arvense*
Garden pea	*P. sativum*

Pea and Vetch Group—Continued

Common name	Scientific name
Austrian Winter pea	*P. sativum* (var. *arvense*)
Common vetch	*Vicia sativa*
Hairy vetch	*V. villosa*
Horsebean	*V. faba*
Narrowleaf vetch	*V. angustifolia*
Purple vetch	*V. atropurpurea*
Monantha vetch	*V. articulata*
Sweet pea	*Lathyrus odoratus*
Rough pea	*L. hirsutus*
Tangier pea	*L. tingitanus*
Flat pea	*L. sylvestris*
Lentil	*Lens culinaris* (*esculenta*)

Cowpea Group

Cowpea	*Vigna sinensis*
Asparagus-bean	*V. sesquipedalis*
Common lespedeza	*Lespedeza striata*
Korean lespedeza	*L. stipulacea*
Sericea lespedeza	*L. cuneata*
Slender bushclover	*L. virginica*
Striped crotalaria	*Crotalaria mucronata*
Sunn crotalaria	*C. juncea*
Winged crotalaria	*C. sagittalis*
Florida beggarweed	*Desmodium tortuosum*
Tick trefoil	*D. illinoense*
Hoary tickclover	*D. canescens*
Kudzu	*Pueraria thunbergiana*
Alyceclover	*Alysicarpus vaginalis*
(No common name)	*Erythrina indica*
Pigeonpea	*Cajanus cajan* (*indicus*)
Guar	*Cyamopsis tetragonoloba*
Jackbean	*Canavalia ensiformis*
Peanut	*Arachis hypogaea*
Velvetbean	*Stizolobium deeringianum*
Lima bean	*Phaseolus lunatus* (*macrocarpus*)
Adzuki bean	*P. angularis*
Mat bean	*P. aconitifolius*
Mung bean	*P. aureus*
Tepary bean	*P. acutifolius* var. *latifolius*
Partridge-pea	*Chamaecrista fasciculata*
Acacia	*Acacia linifolia*
Kangaroo-thorn	*A. armata*
Wild-indigo	*Baptisia tinctoria*
Hairy indigo	*Indigofera hirsuta*

Bean Group

Garden beans, kidney bean, Navy bean, pinto bean.	*Phaseolus vulgaris*
Scarlet Runner bean	*P. coccineus* (*multiflorus*)

Lupine Group

Common name	Scientific name
Blue lupine	*Lupinus angustifolius*
Yellow lupine	*L. luteus*
White lupine	*L. albus*
Washington lupine	*L. polyphyllus*
Sundial	*L. perennis*
Texas bluebonnet	*L. subcarnosus*
Serradella	*Ornithopus sativus*

Soybean Group

All varieties of soybeans.	*Glycine max* (*Soja max*)

It is obviously not necessary to have a specific culture of legume bacteria for every legume to be planted. *It is necessary and extremely important, however, to have a sufficient number of different strains of known effectiveness in an inoculant to inoculate all the legumes specified on the culture label.*

The following legumes appear to require specific strains of legume bacteria for effective inoculation:

Specific Strain Group

Common name	Scientific name
Birdsfoot trefoil	*Lotus corniculatus*
Big trefoil	*L. uliginosus*
Foxtail dalea	*Dalea alopecuroides*
Black locust	*Robinia pseudoacacia*
Trailing wild bean	*Strophostyles helvola*
Hemp sesbania	*Sesbania exaltata*
Kura clover	*Trifolium ambiguum*
Sanfoin	*Onobrychis vulgaris* (*sativus*)
Crown vetch	*Coronilla varia*
Siberian pea-shrub	*Caragana arborescens*
Garbanzo	*Cicer arietinum*
Leadplant	*Amorpha canescens*

It has been known for a long time that nodules do not form on some legumes. Among these are redbud (*Cercis canadensis*), Kentucky coffeetree (*Gymnocladus dioicus*), honeylocust (*Gleditsia triacanthos*), and sicklepod (*Cassia tora*).

Strain Variation

That not all legume bacteria are the same has been repeatedly emphasized. Some prefer certain specific groups of legumes, others only a single species. Some varieties, particularly soybeans and peas, have specific legume bacteria preferences. A strain may produce excellent results on one variety, but it would be a poor nitrogen fixer on another variety.

Another difference is the variation in effectiveness between strains of bacteria isolated from the same legumes and from different legumes within the same group. This type of strain variation among the legume bacteria has great practical significance. As nitrogen fixers, some are high, some are poor, and others show gradations between these extremes.

The search for new and better strains is continuous, for the cultures that prove of greatest benefit under field conditions will be the ones in greatest demand by farmers. Strain variation among legume bacteria is illustrated in figure 8.

Some important observations should be emphasized.

1. The alfalfa and sweetclover strains work on alfalfa or sweetclover equally well, but they fail to produce nitrogen fixation in burclovers and fenugreek. Strains from bur-clover and fenugreek, on the other hand, work and fix nitrogen on bur-clovers, fengugreek, alfalfa, and sweetclovers.

2. Strains from red and white clovers fix nitrogen on their host plants, but not all of them effectively inoculate crimson clover. One strain isolated from berseem clover was effective on all clovers tested except the white and the red.

3. Strains of legume bacteria show definite varietal preferences. For example, some soybean bac-

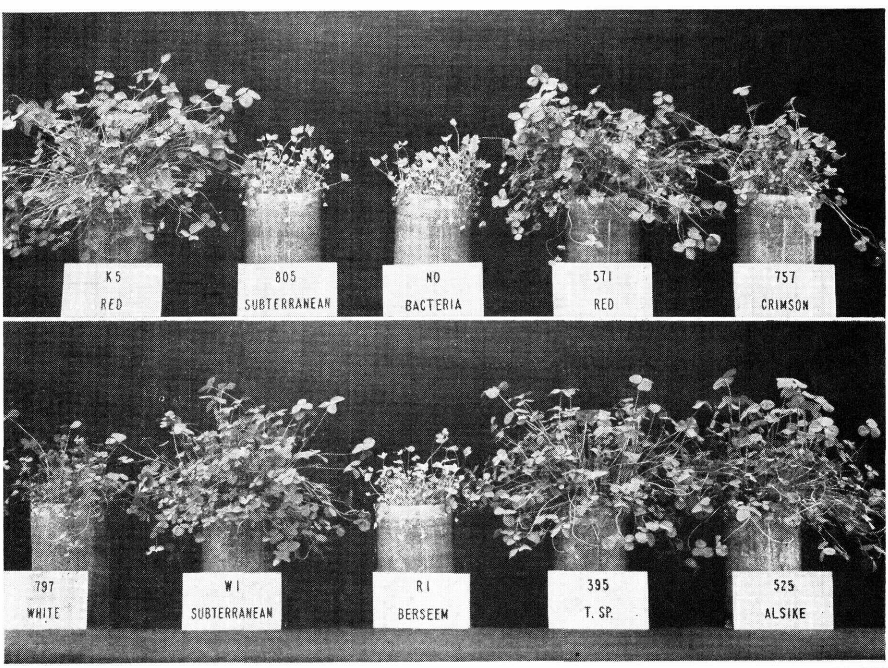

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Figure 8.—**How different strains of clover bacteria affect Ladino clover.**

teria work on one or two soybean varieties better than on others. The same is true for different varieties of canning or freezing peas.

4. Strains of legume bacteria from birdsfoot trefoil may be highly effective on its host but totally ineffective on big trefoil, which is another species. Strains that may be highly effective on big trefoil fail to work on birdsfoot trefoil (fig. 9). However, experimental work has shown that when effective strains for each of these trefoils are mixed together into a single inoculant, both species can be satisfactorily inoculated.

Greenhouse tests of different strains of legume bacteria enable the bacteriologist to select the best strains for a given legume. Field tests of these selected strains are then desirable to see how they affect the legumes when they are grown on the farm (figs. 10 and 11).

Parasitic Strains

The method for studying strain variation has also shown the existence of parasitic strains of legume bacteria. These parasitic, or ineffective, strains enter the root and form numerous small nodules, but fail to fix any nitrogen or otherwise benefit the plant. The nodulation of guar plant roots produced by 1 ineffective strain and by 2 effective strains is shown in figure 12.

This discovery of parasitic strains makes the number of nodules of less importance as a measure of value of a legume inoculant. Numbers of nodules fail to tell the whole story of the effectiveness of the bacteria in fixing nitrogen. It is necessary to measure plant growth responses, particularly mass, vigor, color, and,

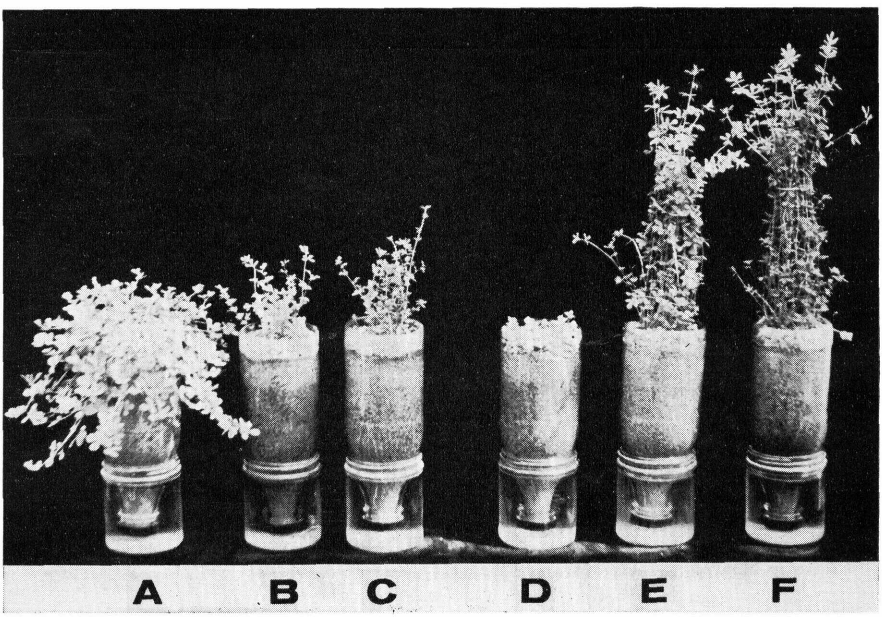

Figure 9.—**Big trefoil bacteria is ineffective on birdsfoot trefoil; birdsfoot trefoil bacteria is ineffective on big trefoil.**

A, **Big trefoil; seed inoculated with big trefoil bacteria.**
B **and *C,* Birdsfoot trefoil; seed inoculated with big trefoil bacteria.**
D, **Big trefoil; seed inoculated with birdsfoot trefoil bacteria.**
E **and *F,* Birdsfoot trefoil; seed inoculated with birdsfoot trefoil bacteria.**

if possible, total nitrogen content. Producers of commercial cultures today realize the great importance of using only highly effective nitrogen-fixing strains of bacteria in the preparation of their legume inoculants. Many bacteria of effective strains added to legume seeds prevent the entrance of ineffective strains already in the soil.

Inspection

To protect farmers from buying worthless cultures, certain control agencies have been set up. In 1916 the United States Department of Agriculture began testing commercial legume inoculants in accordance with an act of Congress providing for soil microbiological investigations. Cultures were procured each

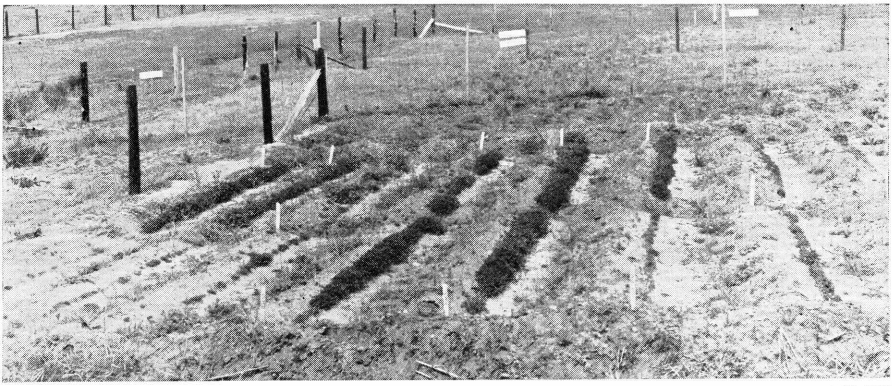

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***Figure 10.*—Effect of different strains of clover bacteria on white clover. Several strains were no better than the uninoculated row shown in right corner.**

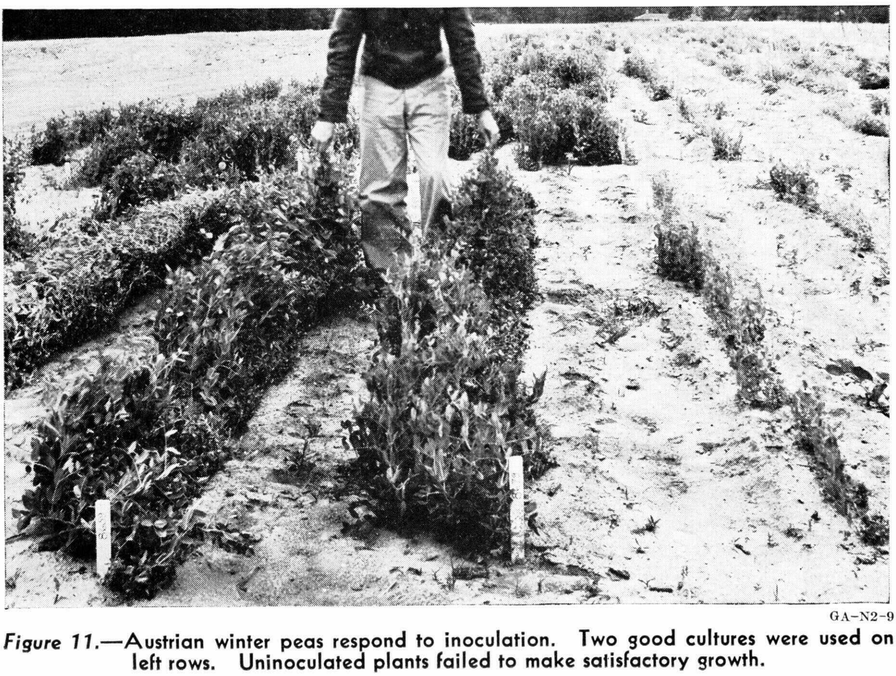

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***Figure 11.*—Austrian winter peas respond to inoculation. Two good cultures were used on left rows. Uninoculated plants failed to make satisfactory growth.**

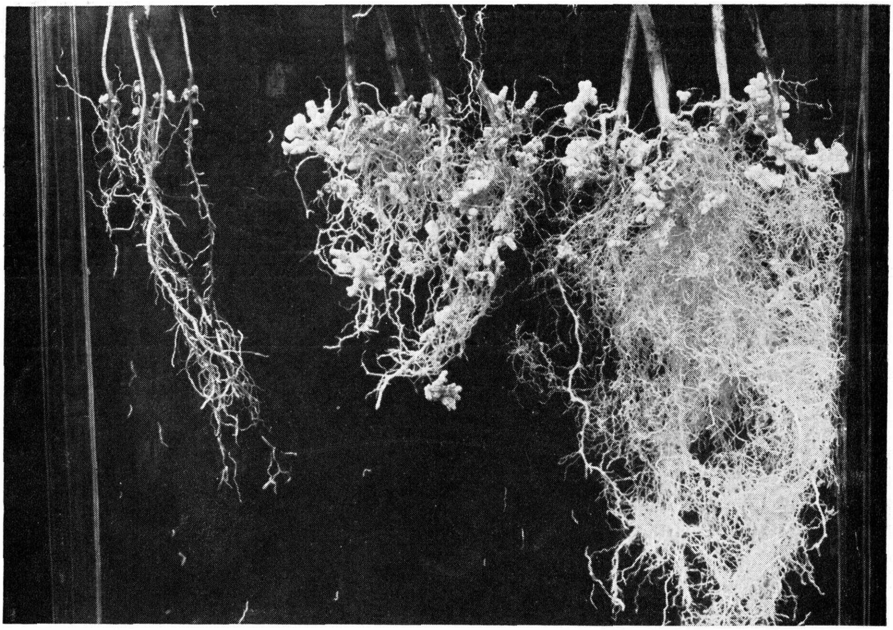

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Figure 12.—**Guar plant roots. Small, parasitic nodules are shown at left. Other nodules were produced by effective strains of bacteria. All plants were grown under the same conditions.**

year in the open market and tested on the legume plants for which they were recommended. From time to time results of these tests were published along with the names of the manufacturers or distributors.

The testing process consists of inoculating seed according to directions on the container and planting them in sterile sand moistened with a sterile nitrogen-free nutrient solution. Great care is taken to prevent entry of legume bacteria and the transfer of bacteria from one seed pot to another. A culture is considered satisfactory if under these conditions it produces nodules, increases plant growth, and produces a plant that is darker green than uninoculated controls. Field tests are sometimes made.

In addition to testing commercial legume inoculants, the Department has kept in close touch with inoculant producers. Its representatives have visited the more important laboratories and offered to help the manufacturers in production problems and thus assure better cultures for the farmer. A few of the States also have control agencies to protect their farmers from unscrupulous producers and dealers. All these agencies have had a decidedly beneficial effect in bringing about improvements and raising the standard and quality of inoculants.

Commercial cultures for legumes have reached a high state of reliability and usually produce satisfactory results.

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